

Attorney's Docket No.: 56446-20010.01/-045US1/D230-1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mathur, et al. Art Unit : 1652
Serial No. : 09/202,681 Examiner : Richard Hutson, Ph.D.
Filed : December 23, 1999
Title : PHOSPHATASES, POLYNUCLEOTIDES ENCODING THEM AND
METHODS OF MAKING AND USING THEM (amended)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Jay Short, am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as CEO and as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

2. I declare that procedures for identifying nucleic acids that encode enzymes such as phosphatases were conventional and routine in the art at the time of the invention. Procedures for identifying polypeptides having phosphatase activity were conventional and routine in the art at the time of the invention. For example, an exemplary assay for identifying polypeptides having phosphatase activity is described in the paragraph spanning pages 39 and 40 of the WO 97/48416 specification. One of ordinary skill in the art using the teaching of the specification could have made and expressed nucleic acids having a percent sequence identity to an exemplary nucleic acid, or, which hybridized under defined conditions to an exemplary nucleic acid, and using routine screening could have determined with predicable positive results which of those nucleic acids encoded a polypeptide having phosphatase activity. Thus, using the teaching of the specification one of ordinary skill in the art would be able to ascertain the scope of the claimed genus of phosphatases and phosphatase-encoding nucleic acids with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing.

Certificate of Transmission by Facsimile

I hereby certify that this correspondence is being transmitted by facsimile to the USPTO on the date indicated below to central fax number 703 872-9306

May 18, 2004
Date of Transmission


Jeanne Armour

sd-199946

Applicant : Mathur, et al.
Serial No. : 09/202,681
Filed : December 23, 1999
Page : 2 of 4

Attorney's Docket No.: 56446-20010.01/-
045US1/D230-1

3. I declare that one of ordinary skill in the art using the teaching of the specification would have been able to make nucleic acids that encode for fragments of the exemplary phosphatase-encoding nucleic acid and express those nucleic acids, and using routine screening determine with predicable positive results which of the identified nucleic acids encode polypeptides having phosphatase activity. One of ordinary skill in the art using the teaching of the specification would have been able to ascertain what fragments of the exemplary nucleic acid could be used to identify phosphatase-encoding nucleic acids. One of ordinary skill in the art using the teaching of the specification would have been able to ascertain what fragments of the exemplary phosphatase could be used to identify enzymatically active fragments. Thus, using the teaching of the specification one of ordinary skill in the art would be able to ascertain the scope of the claimed genus of polypeptides with phosphatase activity and phosphatase-identifying nucleic acids with reasonable clarity and recognize that Applicants' were in possession of the claimed invention at the time of filing.

4. I declare that procedures for modifying and expressing nucleic acids were conventional and routine in the art at the time of the invention. Procedures for determining the activity of the expressed modified nucleic acids and determining if the nucleic acids expressed a polypeptide with phosphatase activity were conventional and routine in the art at the time of the invention. Procedures for determining sequence identity to an exemplary nucleic acid or whether a nucleic acid hybridized to a target nucleic acid under defined conditions were routine in the art at the time of the invention. Procedures for expressing and screening for phosphatase activity were conventional and routine in the art at the time of the invention.

5. I declare that one of ordinary skill in the art using the teaching of the specification would have been able to make phosphatase-encoding nucleic acids having at least 70% sequence identity to the exemplary nucleic acid, or phosphatase-encoding nucleic acids that hybridize under defined hybridization conditions to the exemplary nucleic acid, to make and use the genus of compositions of the invention without undue experimentation. It was considered routine by one skilled in the art at the time of the invention to screen for multiple substitutions or modifications of a nucleic acid or a polypeptide for functional variations, including screening for

sd-199946

Applicant : Mathur, et al.
Serial No. : 09/202,681
Filed : December 23, 1999
Page : 3 of 4

Attorney's Docket No.: 56446-20010.01/-
045US1/D230-1

a genus of phosphatase-encoding nucleic acids or a genus of phosphatases. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that encode enzymatically active fragments of an exemplary enzyme. It was considered routine by one skilled in the art at the time of the invention to screen for subsequences of nucleic acids that can identify by hybridization a phosphatase-encoding nucleic acid. For example, high through-put methods for screening for enzyme activity, such as phosphatase activity, were well known in the art. While the numbers of samples needed to be screened may have been high, the screening procedures were routine and successful results (e.g., finding a genus of nucleic acids encoding phosphatases) predictable. At the time of the invention it would have been considered routine by one skilled in the art to generate and screen multiple substitutions or multiple modifications in an exemplary nucleic acid sequence and predictably generate a genus of phosphatase-encoding nucleic acids or a genus of phosphatases.

6. I declare that it was not necessary for the skilled artisan to understand which specific regions of phosphatase sequence or structure needed to be modified without affecting function or activity to routinely generate the claimed genus of phosphatase-encoding nucleic acids. Methods for sequence modifications were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate phosphatase-encoding sequences without need of knowing which specific regions of phosphatase sequence or structure affected phosphatase function or activity. Methods known at the time of the invention for modifying nucleic acid sequences in combination with high through-put enzyme activity screening known at the time of the invention, made methods that require previous knowledge of protein structure, including secondary or tertiary structure, active site sequences, and the like obsolete and unnecessary. Accordingly, using methods known in the art at the time of the invention it would not have been necessary to understand which specific regions of phosphatase structure needed to be modified to generate a genus of nucleic acids or polypeptides for practicing the invention without undue experimentation.

7. I declare that the specification provides sufficient guidance to one of ordinary skill in the art as to whether a nucleic acid or polypeptide falls within the scope of the claimed

sd-199946

Applicant : Mathur, et al.
Serial No. : 09/202,681
Filed : December 23, 1999
Page : 4 of 4

Attorney's Docket No.: 56446-20010.01/-
045US1/D230-1

genus. Methods for determining the requisite structure (sequence based on percent sequence identity to an exemplary nucleic acid or polypeptide) and function (phosphatase activity) are clearly set forth in the specification. At the time of the invention, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and enzyme activity screening protocols were well known in the art. The specification sets forth an exemplary phosphatase screening assay to determine if a nucleic acid or polypeptide is within the scope of the claimed genus, *inter alia*, in the paragraph spanning pages 39 and 40 of the WO 97/48416 specification. Methods for determining sequence identity were also routine and well known in the art at the time of the invention. While the numbers of alternative species that needed to be screened may have been high, the protocols for screening were routine and positive results predictable. Accordingly, the specification provided sufficient guidance to one of ordinary skill in the art to make and use the claimed genus of nucleic acids or polypeptides to practice the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: _____

Jay Short

sd-199946

CURRICULUM VITAE**NAME:** Jay M. Short, Ph.D.**EDUCATION:**

1981 - 1985	Ph.D., Biochemistry Case Western Reserve University, Cleveland, Ohio
1980 - 1981	Graduate Study, Macromolecular Science Case Western Reserve University, Cleveland, Ohio
1976 - 1980	B.A. with Honors, Chemistry Taylor University, Upland, Indiana

RESEARCH & PROFESSIONAL EXPERIENCE:

<u>1999 - present</u>	CEO, President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1998 - present</u>	President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1997 - 1998	Executive Vice President, Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
1994 - 1997	Chief Technology Officer & Board of Directors Diversa Corporation San Diego, California
<u>1990 - 1994</u>	President Stratacyte, Inc. La Jolla, California
<u>1992 - 1994</u>	Vice President R&D (Research) and Operations Stratagene Cloning Systems La Jolla, California
1989 - 1992	Vice President R&D (Research) and Biological Operations Stratagene Cloning Systems
1988 - 1989	Senior Staff Scientist Research and Development Stratagene Cloning Systems

1985 - 1988**Staff Scientist**
Research and Development
Stratagene Cloning Systems**1981 - 1985****Ph.D. Research**
Case Western Reserve University
Dr. Richard W. Hanson's Laboratory,
Identification and characterization of the promoter for
P-enolpyruvate carboxykinase.
First identification of a cAMP regulatory domain.**1980 - 1981****Graduate Student Research**
Case Western Reserve University
Dr. Bruce Roe's Laboratory, Analysis of the cellulase activity of *Trichoderma viride*.**TEACHING EXPERIENCE:**

Thesis Advisor (1988-1993), University of Uppsala, Sweden, Ph.D. for Michelle Alting-Mees
Lecturer (1992), Committee for Advanced Scientific Education, Center for Drug
Evaluation and Research, FDA.
Faculty (1989), Transgenic Mouse Model and Its Application in Assessing
In Vivo Mutagenesis, Genetic Toxicology Workshop (3rd Annual).
Microbiological Associates Inc. Bethesda, MD.
Faculty (1987), DNA Cloning and Expression. Physiology Society Workshop. San Diego, CA.
Teaching Assist., (1981-1985). Molecular and Cellular Biology. Case Western
Reserve University.
Teaching Assist., (1981). Physiological Chemistry. Kent State Univ., Kent, OH.
Teaching Assist., (1978-1980). Quantitative Analysis. Taylor University.

AWARDS, PROFESSIONAL MEMBERSHIPS, ACCOMPLISHMENTS, AND ACTIVITIES:

Visiting Scientist, International Centre of Insect Physiology and Ecology (ICIPE), Kenya (2002-2004)
Science & Technology Committee, *BIOCOM San Diego*
Advisory Board, IngleWood Ventures
Finalists for UCSD Connect's Most Innovative New Product Award in the Biotechnology R&D Category
Advisory Board, *Chemical & Engineering News*
Board of Advisors and Founding Member, Division of Biological Sciences, *UCSD*
Board Director, *BIOCOM San Diego*
Chairman of the Board, Innovase
Board Director, Zymetrics
Board Director, Innovase
Director at Large, *YPO (Young Presidents' Organization) San Diego*.
2001 T-Sector Life Science Innovator Award.
2001 Deloitte and Touche's Orange County / San Diego 2001 Technology "Fast 50".
San Diego Entrepreneur of the Year 2001.
YPO (Young Presidents' Organization) San Diego.
YPO (Young Presidents' Organization) International.
Finalist for San Diego Entrepreneur of the Year in 2000.
Largest Biotechnology IPO raising over \$200MM.
Founding management member of Diversa Corporation.
Panel for Chemical Science & Technology for NIST, appointed by the National Research Council (1997-2000).
Chairman (1993), Discussion Group, Society of Toxicology Conference.
U.S. Committee Member for Evaluation of Biotechnology Research in Spain.
Editor, *Mutation Research*.

UCSD Connect Program (1991) 1st Place Award for Innovation and Entrepreneurship in Biotechnology (over 50 competing biotech companies).
 UCSD Connect Program (1990) 1st Place Award for Innovation and Entrepreneurship in Biotechnology.
 Consultant for European Economic Community on Transgenic Toxicology Testing (91-94).
 The New York Academy of Sciences.
 Reviewer for *Proceedings of the National Academy of Sciences, Genetic Analysis Techniques, Analytical Biochemistry, & Nucleic Acids Research*.
 American Association for the Advancement of Science.
 American Chemical Society.
 American Society of Biochemistry and Molecular Biology.
 American Society of Microbiology.
 Environmental Mutagenesis Society.
 Society for Industrial Microbiology
 Society of Toxicology.
 Japanese Environmental Mutagen Society.
 Who's Who Registry of Business Leaders (1994-1995)
 American Men and Women of Science (1995)
 NIEHS Peer Review Committee.
 SBIR Study Section.
 SBIR Annual Report (1993) Program Success Profile (Top 8 of 800 Companies).
 Stratagene (1990) Innovation Award - Lambda ZAP[®] vector.
 Stratagene (1990) Service Award
 Stratagene (1991) Innovation Award - Big Blue[®] Transgenic Testing System.
 Stratagene (1992) Most Innovative Award - Managers/Supervisors.
 Institutional Animal Care and Use Committee (IACUC), Chairman and Institutional Official.
 Award from the University of Victoria for Contributions to the Development of Short-term Transgenic Mutation Assays.
 Nominated as Council Member for the U.S. Environmental Mutagen Society.
 Board Director, *Stressgen (TSE), Victoria, BC, Canada*
 Board Director & Treasurer, *Stressgen Therapeutics, Victoria, BC, Canada*
 Board Director & Secretary, *Stressgen Therapeutics, Victoria, BC, Canada*
 Board Director, *Diversa, La Jolla, CA*
 Board Director, *Invitrogen, Carlsbad, CA*
 Consultant, *Stratagene Cloning Systems, La Jolla, CA*
 Consultant, *Micro Product Systems, Lynn, IN*
 Reviewer for U.S. Congressional Office of Technology Assessment (OTA) on *The Human Genome Project and Patenting DNA Sequences*.

MEDIA:

ABC Discovery News, ABC San Diego Channel 10, BBC Radio, Bioinformed Newsletter, Biotechnology Newsletter, BioVentures View, Business Daily, Business Week, CEO Cast, Chemical Engineering, Chemical Week, Chemistry & Industry (UK), CNBC, CNN Science & Technology, dBusiness.com, Discovery Magazine, Forbes.com, Good Morning America, Horizon Air Magazine, Idea TV, Inside Business Radio Show, JAG Financial News, Los Angeles Times, NBC San Diego Channel 7/39, National Radio Report, New York Times, Pirateinvestor.com, R&D Magazine, RTL German Television, Reuters, San Diego Business Transcript, San Diego Channel KUSI, San Diego Channel 10, San Diego Magazine, San Diego Union Tribune, Scientist, Time Magazine, The Discovery Channel, The Motley Fool, Time Magazine, USA Today, Wall Street Journal, Wall Street Transcript, Washington Post

PATENTS:

DNA Cloning Vectors with *in vivo* Excisable Plasmids (1987).
 Mutagenesis Testing Using Transgenic Animals Carrying Marker Genes (1987).
 Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test

DNA Sequences (1987).
Dietary and Hormonal Regulation of Expression of Exogenous Genes in Transgenic Animals Under Control of the Promoter of the Gene Phosphoenolpyruvate Carboxykinase (1988).
A Transgenic Mouse for Measurement and Characterization of Mutation Induction *In Vivo* (1989).
Rapid Screening Mutagenesis and Teratogenesis Assay (1989).
A Combinatorial Approach to Regenerating the Immunoglobulin Repertoire in Prokaryotic Cells (1990).
Transgenic Animal Models for *In Vivo* Mutagenesis Testing (1990).
Polycos Vectors (1991).
A Lambda Packaging Extract Lacking β -Galactosidase Activity (1991).
A System for Regulation of Eukaryotic Genes (1991).
Methods for Phenotype Creation from Multiple Gene Populations (1991).
Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Mutagenesis Testing Using Transgenic Non-Human Animals Carrying Test DNA Sequences (1992).
Selectable System Patent (1992).
Polycos Mutagenesis Systems (1993).
Use of Trans-acting Proteins for the Development of an *In Situ* Expression Screening System (1993).
Enzyme Kits and Libraries (1995).
Enzyme Activity Screening of Clones having DNA from Uncultivated Microorganisms (1995).
Enzyme Tiered (1995).
Method for Screening for Enzyme Activity (1995).
Combined Enzyme Screening/Evolution (1995).
Uncultured/Activity Screening (1995).
Directed Evolution of Thermophilic Proteins (1995).
Combinatorial Enzyme Development (Directed Mutagenesis) (1996).
Protein Activity Screening of Clones having DNA from Uncultivated Microorganisms (1996).
Production and Use of Normalized DNA Libraries (1996).
Methods of DNA Shuffling with Polynucleotides Produced by Blocking or Interrupting a Synthesis or Amplification Process (1996).
Method of Screening for Enzyme Activity (Biopanning) (1996).
Directed Evolution of Thermophilic Enzymes (1996).
Environmental Biopanning (1996).
Combinatorial Enzyme Development (1996).
Enzyme Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1996).
Normalized Samples/Libraries (1996).
Reassembled Pools of Mutagenized DNA & Procedure (1996).
Fluorescent-based Single Screening for Enzymes (1996).
High Throughput Screening for Novel Enzymes (1997).
Nucleotide Sequence of the *Aquifex aeolicus* Genome, Fragments Thereof, and Uses Thereof (1997).
Screening for Novel Bioactivities (1997).
Screening for Novel Compounds which Regulate Biological Interactions (1997).
Method for Screening Enzyme Activity (1997).
High Throughput Screening for Novel Enzymes (1997).
"Discovery" (whole process, including uncultivated, normalized, biopanning, screening, evolving, (etc.) (1997).
Production of Enzymes Having Desired Activities By Mutagenesis (1999).
Protein Activity Screening of Clones Having DNA from Uncultivated Microorganisms (1999).
Method of DNA Reassembly by Interrupting Synthesis (1999).
Production and Use of Normalized DNA Libraries (1999).
Enzyme Kits and Libraries (1999).
Screening for Novel Bioactivities (2000).
Method for Screening for Enzyme Activity (2000).
Screening for Novel Bioactivities (2000).
Production and Use of Normalized DNA Libraries (2000).
Method of Screening for Enzyme Activity (2000).
Screening Methods for Enzymes and Enzyme Kits (2001).
Saturation Mutagenesis in Directed Evolution (2001).
High Throughput Screening for Novel Enzymes (2001).

Recombinant Bacterial Phytases and Uses Thereof (2001).
 Methods Useful for Nucleic Acid Sequencing Using Modified Nucleotides Comprising Phenylboronic Acid (2001).
 End Selection in Directed Evolution (2001)
 Gene Expression Library Produced From DNA From Uncultivated Microorganisms and Method for Making the Same (2001)
 Directed Evolution of Thermophilic Enzymes (2002)
 Method for Screening for Enzyme Activity (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 End Selection In Directed Evolution (2002)
 Exonuclease-Mediated Gene Assembly in Directed Evolution (2002)
 Screening for Novel Bioactivities(2002)
 Method of DNA Shuffling with Polynucleotides Produced or Blocking or Interrupting Synthesis or Amplification Process (2002)
 Production and Use of Normalized DNA Libraries (2002)
 Sequence Based Screening (2002)
 Non-Stochastic Generation of Genetic Vaccines (2002)
 Over 100 Additional Pending Patent Applications Worldwide.

GRANTS AND CONTRACTS:

- *Phase I Small Business Contract #N43-Am-62282. 1985 - 1986. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase II Small Business Contract #N43-Am-62282. 1988 - 1990. P.I.
Vectors and Techniques for Rapid DNA Sequencing.
- *Phase I Small Business Grant 2R43ES04484-02. 1986 - 1987. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *Phase II Small Business Grant 2R43ES04484-02. 1989 - 1992. P.I.
Identification of Genetic Lesions Leading to Mutations.
- *1R01-ES04728-01A1. 1989 - 1992. (NIEHS) P.I.
Animal Model for Identification of Genetic Lesions.
- *Phase I Small Business Grant #R43GM42291-01. 1989. P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *RFP NIH-ES-88-11. 1989-1994. (NIEHS) Co-I.
Development of Mutagenesis Assays Using Transgenic Mice.
- *Phase II Small Business Grant #2R44GM42291-02. 1990-1992. (DRG/NIH) P.I.
Switch Mechanism for Gene Expression in Transgenics.
- *Phase I Small Business Grant #1R43GM46585-01. 1991. (DRG/NIH) P.I.
Generation of a Peptide Screening System Through the Development of
Combinatorial-splicing "Polycos" Vectors.
- *Phase I Small Business Grant #1R43CA57066-01. 1992. (NCI) P.I.
Transgenic Rats: A Short-term Mutagenicity Assay for Multi-species Testing of Suspected Human Carcinogens.
- *Phase I Small Business Grant #1R43GM48300-01. 1992. (DRG/NIH) P.I.
Analysis of the Immunoglobulin Hypermutator Mechanism.
- *Phase I Small Business Grant #1R43ES06146-01. 1992. (NIEHS) P.I.
Selectable "Polycos" Shuttle Vectors for In Vivo Mutagenicity Testing.
- *Phase II Small Business Grant #2R44GM46585-02. 1992-1994. (NIGMS) P.I.
Peptide Screening Utilizing Combinatorial Polycos Vector.
- *Phase I Small Business Grant #1R43RR08667-01. 1992-1993. (DRG/NIH) Co-I.
A One-step PCR Cloning System Based on FLP Recombination.
- *Phase II Small Business Grant #2R44CA57066-02. 1993-1995. (NCI) P.I.
Transgenic Rats:Transgenic Rat Model for Mutagenicity Testing.
- *Phase I Small Business Grant. 1993-1994. (NIH) Co-I.
Transgenic Fish Model for Mutagenicity Testing.
- *Phase II Small Business Grant (1994-1996). (NIH) P.I.
"Polycos" Shuttle Vectors for Mutagenicity testing.
- *Phase I Small Business Grant. 1994. (NIH) Co-I.
Vector System for Studying Protein-Protein Interactions.

- *CRADA with LLNL. 1994. (NIH) Co-I.
Mouse and Rat Painting Probes.
- *CRADA with FDA. 1994. (NIH) Co-I.
Tamoxifen Testing in F-344 Rats.
- *CRADA with NASA. 1994. (NIH) Co-I.
Radiation Damage in the Microgravity Environment.

ABSTRACTS AND INVITED LECTURES:

Over 200 Abstracts and Invited Lectures.

PUBLICATIONS:

1. Yoo-Warren, H., Monahan, J.E., Short, J.M., Short, H., Bruzel, A., Wynshaw-Boris, A., Meisner, H.M., Samols, D., and Hanson, R.W. (1983) Isolation and Characterization of the Gene Coding for Cytosolic Phosphoenolpyruvate Carboxykinase (GTP) from the Rat. *Proc. Natl. Acad. Sci. U.S.A.*, 80:3656-3660.
2. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1984) Identification of cAMP Regulatory Region in the Gene for Rat Cytosolic Phosphoenolpyruvate Carboxykinase (GTP): Use of Chimeric Genes Transfected into Hepatoma Cells. *J. Biol. Chem.*, 259:12161-12169.
3. Wynshaw-Boris, A., Lugo, T.G., Short, J.M., Fournier, R.E.K., and Hanson, R.W. (1985) A Region of the Gene for Rat Cytosolic P-enolpyruvate Carboxykinase Confers cAMP Responsiveness to the HSV-thymidine Kinase Gene. In: *Membrane Receptors and Cellular Recognition*, (M. Czech and C.R., Kahn, eds.), Alan Liss Inc., New York, pp 339-346.
4. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. I. Multiple Hormone Regulatory Elements and the Effects of Enhancers. *J. Biol. Chem.*, 261:9714-9720.
5. Short, J.M., Wynshaw-Boris, A., Short, H.P., and Hanson, R. W. (1986) Characterization of the Phosphoenolpyruvate Carboxykinase (GTP) Promoter-Regulatory Region. II. Identification of cAMP and Glucocorticoid Regulatory Domains. *J. Biol. Chem.*, 261:9721-9726.
6. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1986) The Determination of Sequence Requirements for Hormonal Regulation of Gene Expression. *Biotechniques*, 4:104-119.
7. Burns, D.M., Bhandari, G., Short, J.M., Sanders, P.G., Wilson, R.H., and Miller, R.E. (1986) Selection of a Rat Glutamine Synthetase cDNA Clone. *Biochemical and Biophysical Research Communications*, 134:146-151.
8. Hod., Y. Cook, J.S., Weldon, S.L., Short, J.M., Wynshaw-Boris, A., and Hanson, R.W. (1986) Differential Expression of the Genes for the Mitochondrial and Cytosolic Forms of P-enolpyruvate Carboxykinase Gene. In: *Metabolic Regulation: Application of Recombinant DNA Techniques*, (A.G., Goodridge and R.W. Hanson eds.), Annals of the New York Academy of Sciences, New York, Vol. 278, pp. 31-45.
9. Wynshaw-Boris, A., Short, J.M., and Hanson, R.W. (1987) cis - acting Regulatory Elements in Hormonally Responsive Genes. In: *Progress in Nucleic Acid Research and Molecular Biology* (W.E. Cohn and K. Moldave eds.), Academic Press, Inc., Orlando, Florida, 34:59-87.
10. Bullock, W., Fernandez, J.M., and Short, J.M. (1987) XL1-Blue: A High Efficiency Plasmid Transforming *recA E.coli* Strain With β -Galactosidase Selection. *Biotechniques*, 5:60-64.
11. Short, J.M., Fernandez, J.F., Sorge, J.A., and Huse, W. (1988) Lambda ZAP[®]: A Bacteriophage Lambda Expression Vector With *In Vivo* Excision Properties. *Nucleic Acids Res.*, 16:7583-7600.

12. Short, J.M. (1988) Book Review: Vectors - A Survey of Molecular Cloning Vectors and Their Uses. Raymond L. Rodriques and David T. Denhardt, eds, Butterworths, Stoneham, MA. *Genomics*, 2:270-271.
13. Short, J.M., and Pollard, A. (1988) Gigapack XL: Size Selective Packaging Extract. *Strategies in Mol. Biol.*, 1:5-7.
14. Kretz, P.L., and Short, J.M. (1989) Gigapack II: A Restriction Deficient (*mcrA*-, *B*-, *hsd*-, *mrr*-) Lambda Packaging Extract. *Strategies in Mol. Biol.*, 2(2):25-26.
15. Kretz, P.L., Reid, C.H., Greener, A., and Short, J.M. (1989) Effect of Lambda Packaging Extract Mcr Restriction Activity on DNA Cloning. *Nucleic Acids Res.* 17:5409.
16. Sastry, L., Alting-Mees, M., Huse, W.D., Short, J.M., Sorge, J.A., Hay, B.N., Janda, K.D., Benkovic, S.J., and Lerner, R.A. (1989) Cloning of the Immunological Repertoire in *E. coli* for Generation of Monoclonal Catalytic Antibodies I. Construction of a V_H Specific cDNA Library. *Proc. Natl. Acad. Sci. U.S.A.*, 86:5728-5732.
17. Short, J.M. (1989) The Use of Lambda Phage Shuttle Vectors in Transgenic Mice for Development of a Short Term Mutagenicity Assay. In: *Fifth International Conference on Environmental Mutagens*, Alan Liss, Inc., New York, Part A:335-367. Article and Lecture.
18. Alting-Mees, M., and Short, J.M. (1989) pBluescript II: Gene Mapping Vectors. *Nucleic Acids Res.*, 17:9494.
19. Shopes, B., Alting-Mees, M., Amber, J.R., Ardourel, D., Callahan, M., Detrick, J., Hay, B.N., Hogrefe, H.H., Greener, A., Gross, E.A., Kubitz, M.M., Mullinax, R.L., Wilson, C., Short, J.M., and Sorge, J.A. (1990) Bacteriophage Immuno-expression Libraries: An Emerging Technology for the Identification and Production of Monoclonal Antibodies. *Antibody Engineering, New Tech. & Application Implications*. pp. 98-101.
20. Alting-Mees, M., Amberg, J., Ardourel, D., Elgin, E., Greener, A., Gross, E.A., Kubitz, M., Mullinax, R.L., Short, J.M., and Sorge, J.A. (1990) Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas. *Strategies in Mol. Biol.*, 3:1-9.
21. Kohler, S., Provost, S., Dyaico, M., Sorge, J., and Short, J.M. (1990) Development of a Short-term, *In Vivo* Mutagenesis Assay: The Effects of Methylation on the Recovery of a Lambda Phage Shuttle Vector from Transgenic Mice. *Nucleic Acids Res.*, 18:3007-3013.
22. Kohler, S., Provost, G.S., Kretz, P.L., Fieck, A., and Short, J.M. (1990) An *In Vivo* Assay Using Transgenic Mice to Analyze Spontaneous and Induced Mutations at the Nucleic Acid Level. *Strategies in Mol. Biol.*, 3:19-21.
23. Kretz, P., Kohler, S., and Short, J.M. (1990) The Effect of *E. coli* Minute 98 Locus on DNA Containing Eukaryotic Modifications. *Strategies in Mol. Biol.*, 3:21-22.
24. Mullinax, R.L., Gross, E.A., Amberg, J., Hogrefe, H., Kubitz, M., Greener, A., Alting-Mees, M., Ardourel, D., Hay, B.N., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Identification of Human Antibody Fragment Clones Specific for Tetanus Toxin in a Bacteriophage Lambda Immuno-Expression Library. *Proc. Natl. Acad. Sci. U.S.A.*, 87:8095-8099.
25. Cline, J., Lundberg, K., Nielson, K., Sorge, A., Short, J.M., and Mathur, E.J. (1990) StrataClean Resin: Non-Toxic Protein Extraction. *Strategies in Mol. Biol.*, 4(4):49-51.
26. Mullinax, R.L., Gross, E.A., Amber, J.R., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., Sorge, J.A., and Shopes, B. (1990) Human Antibody Clones Isolated From a Bacteriophage Lambda Immunoexpression Library. *Strategies in Mol. Biol.*, 4(4):51-52.
27. Provost, G.S., Kohler, S.W., Fieck, A., Kretz, P.L., Molina, T., and Short, J.M. (1990) Short-term Germ Line and Somatic Cell Mutagenesis Testing With *LacI* Lambda Phage Shuttle Vectors in Transgenic Mice. *Strategies in Mol. Biol.*, 4(4):55-56.

28. Kohler, S.W., Provost, G.S., Kretz, P.L., Fieck, A., Sorge, J.A., and Short, J.M. (1990) The Use of Transgenic Mice for Short Term, *In Vivo* Mutagenicity Testing. *Genetic Analysis Techniques*, 7(8):212-218.
29. Shopes, B., Mullinax, R.L., Amber, J.R., Gross, E.A., Hay, B.N., Hogrefe, H.H., Kubitz, M.M., Greener, A., Alting-Mees, M., Ardourel, D., Short, J.M., and Sorge, J.A. (1990) ImmunoZAP/E Bacteriophage Libraries: A New Technology for the Identification and Expression of Monoclonal Antibodies. *Biotech USA Conference Proceedings*, pp.332-341.
30. Raleigh, E.A., Benner, J., Bloom, F., Braymer, H.D., DeCruz, E., Dharmalingam, K., Heitman, J., Noyer-Weidner, M., Piekarowicz, A., Kretz, P.L., Short, J.M., and Woodcock, D. (1991) Nomenclature Relating to Restriction of Modified DNA in *Escherichia coli*. *Journal of Bacteriology*, 173(8):2707-2709.
31. Kretz, P., Kohler, S., and Short, J.M. (1991) Identification and Characterization of a Gene Responsible for Inhibiting Propagation of Methylated DNA Sequences in *mcrA*, *mcrB1* *E. coli* Strains. *Journal of Bacteriology*, 173:4707-4716.
32. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, B., Sorge, J. A., Putman, D., and Short, J.M. (1991) Spectra of Spontaneous and Induced Mutations Using a Lambda ZAP® *LacI* Shuttle Vector in Transgenic Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 88(18):7958-7962.
33. Wyborski, D., and Short, J.M. (1991) Analysis of Inducers of the *E.coli* *Lac* Repressor System in Mammalian Cells and Whole Animals. *Nucleic Acids Research*, 19:4647-4653.
34. Lundberg, K.L., Shoemaker, D.D., Adams, M.W.W., Short, J.M., Sorge, J.A., and Mathur, E.J. (1991) High Fidelity Amplification With a Thermostable DNA Polymerase Isolated from *Pyrococcus Furiosus*. *Gene*, 108:1-6.
35. Kohler, S.W., Provost, G.S., Fieck, A., Kretz, P.L., Bullock, W.O., Putman, D.L., Sorge, J.A., and Short, J.M. (1992) Analysis of Spontaneous and Induced Mutations in Transgenic Mice Using a Lambda ZAP®/*LacI* Shuttle Vector. *Environmental and Molecular Mutagenesis*, 18:316-321.
36. Fieck, A., Wyborski, D., and Short, J.M. (1992) Modifications of the *E. coli* *Lac* Repressor for Expression in Eukaryotic Cells: Effects of Nuclear Signal Sequences on Protein Activity and Nuclear Accumulation. *Nucleic Acids Research*, 20:1785-1791.
37. Hay, B., and Short, J.M. (1992) ExAssist™ Helper Phage and SOLR™ Cells for Lambda ZAP® II Excisions. *Strategies in Mol. Biol.*, 5:16-18.
38. Short, J.M. (1992) Tissue Specific Mutagenesis in Transgenic Mice. *The Toxicology Forum, 1992 Annual Winter Meeting*, pp.79-109.
39. Alting-Mees, M.A., Sorge, J.A., and Short, J.M. (1992) pBluescript II: Multifunctional Cloning and Mapping Vectors. *Methods in Enzymology*, 216:483-495.
40. Short, J.M., and Sorge, J.A. (1992) *In Vivo* Excision Properties of Bacteriophage Lambda ZAP® Expression Vectors. *Methods in Enzymology*, 216:495-508.
41. Short, J.M. (1992) Transgenic Animals for Carcinogenicity and Genotoxicity Testing. *Biotechnology International, The Global Review of Industry Manufacture and Application 1992*. Section.2. pp. 91-99.
42. Provost, G.S., Hamner, R., Kretz, P.L., and Short, J.M. (1992) Response to the Commentary Article: Comparison of Mutation Frequencies Obtained Using Transgenes and Specific-locus Mutation Systems in Male Mouse Germ Cells. *Mutation Research*, 298:145-147.
43. DuCoeur, L.C., Wyborski, D.L., and Short, J.M. (1992) Control of Gene Expression in Eukaryotic Cells Using the *Lac* Repressor System. *Strategies in Mol. Biol.*, 5(3):70-72.
44. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1992) XL1-Blue MRF: *McrA*-, *McrCB*-, *Mrr*-, *HsdRMS*- derivative of XL1-Blue. *Strategies in Mol. Biol.*, 5(3):81-83.

45. Alting-Mees, M., Hoener, P., Ardourel, D., Sorge, J., and Short, J.M. (1992) ZAP Express™ and pBK-CMV, pBK-RSV Phagemid Vectors for Prokaryotic and Eukaryotic Expression. *Strategies in Mol. Biol.*, 5(3):58-61.
46. Short, J.M., Provost, G.S., Kretz, P.L., and Dyaico, M.J. (1992) Overview of the Big Blue® *In Vivo* Mutagenesis Assay. *Mammalian Mutagenesis Study Group Communication – JEMS.MMS*, 6:73-89.
47. Lundberg, K.S., Kretz, P.L., Provost, G.S., and Short, J.M. (1993) The Use of Selection in Recovery of Transgenic Targets for Mutation Analysis. *Mutation Research Letters*, 301/2:99-105.
48. Mirsalis, J., Provost, G.S., Matthews, C., Hamner, R., Schindler, J.E., O'Loughlin, K., MacGregor, J.T., and Short, J.M. (1993) Induction of Hepatic Mutations in *LacI* Transgenic Mice. *Mutagenesis*, 8:265-271.
49. Alting-Mees, M.A., Vaillancourt, P., and Short, J.M. (1993) Phagemids and Other Hybrid Vectors. In: *Plasmids: A Practical Approach*. (ed. K. Hardy). IRL Press, pp. 197-223.
50. Provost, G.S., Kretz, P.L., Dyaico, M., Lundberg, K., and Short, J.M. (1993) Transgenic Systems for *In Vivo* Mutation Analysis. *Mutation Research*, 288:133-149.
51. Hedden, V., Callen W., Short, J.M., and Kretz, K. (1993) Improved Sequence Analysis of Mutations Identified With the Big Blue® System. *Strategies in Mol. Biol.*, 6:27-28.
52. Jerpseth, B., Greener, A., Short, J.M., Viola, J., and Kretz, P.L. (1993) New Restriction-Minus Derivatives of XL1-Blue *E. coli* Cells. *Strategies in Mol. Biol.*, 6:24.
53. Vaillancourt, P., Wyborski, D.L., and Short, J.M. (1993) The FLASH® CAT Kit: A Fast, Sensitive CAT Assay Without Radioactivity. *Strategies in Mol. Biol.*, 5:17-19.
54. Short, J.M., Dyaico, M.J., Provost, G.S., Kretz, P.L., Rogers, B.J., Ardourel, D., Wyborski, D.L. and Moores, J.C. (1993) Transgenic Mice and Rats for Tissue Specific Mutation Analysis. *JEMS*, 22:45-46.
55. Alting-Mees, M.A., and Short, J.M. (1993) Polycos Vectors: Filamentous Phage Packaging Using Lambda Extracts. *Gene*, 137:93-100.
56. Piegorsch, W.W., Lockhart, A.C., Margolin, B.H., Tindall, K.R., Gorelick, N.J., Short, J.M., Carr, G.J., and Shelby, M.D. (1994) Sources of Variability in Data from a *lacI* Transgenic Mouse Mutation Assay. *Environmental & Molecular Mutagenesis*, 23:17-31.
57. Dyaico, M., Provost, G.S., Kretz, P.L., Ransom, S.L., Moores, J.C. and Short, J.M. (1994) The Use of Shuttle Vectors for Mutation Analysis in Transgenic Mice and Rats. *Mutation Research*, 307:461-478.
58. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., and Short, J.M. (1994) Rat2 Cell Line for *In Vitro* Mutagenicity Testing. *Strategies in Mol. Biol.*, 7(2):55-56.
59. Provost, G.S. and Short, J.M. (1994) Characterization of Mutations Induced by Ethylnitrosourea in Seminiferous Tubule Germ Cells of Transgenic B6C3F1 Mice. *Proc. Natl. Acad. Sci. U.S.A.*, 91:6564-6568.
60. Kretz, P.L., Wells, S., and Short, J.M. (1994) Gigapack III: A Single Tube *In Vitro* Lambda Packaging Extract. *Strategies in Molecular Biology* 7:44-45.
61. Knoll, A., Jacobson, D., Kretz, P., Lundberg, K., Short, J., and Sommer, S. (1994) Tissue-Specific Patterns of Spontaneous *LacI* Mutations Recovered From Transgenic Mice. *Mutation Research* 311:57-67.
62. Ashby, J., Short, J.M., Jones, N.J., Lefevre, P.A., Martin, E, Parry, J.M., Burnette, K., Glickman, B.W., and Tinwell, H. (1994) Mutagenicity of o-anisidine to the bladder of *lacI*-transgenic B6C3F1 mice: Absence of ¹⁴C or ³²P bladder DNA adduction. *Carcinogenesis* 15:2291-2296.
63. Alting-Mees, M. and Short, J.M. (1994) Rapid Excision Systems. *Strategies in Molecular Biology* 7(3):70-72.

64. Snead, M., Kretz, P.L., Short, J.M. (1994) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual* H1:1-19.
65. Rogers, B., Provost, G.S., Young, R., Putman, D.L., and Short, J.M. (1995) Intralaboratory Optimization and Standardization of Mutant Screening Conditions Used for a Lambda/LacI Transgenic Mouse Mutagenesis Assay (I). *Mutation Research* 327:57-66.
66. Young, B., Rogers, B., Provost, G.S., Short, J.M., and Putman, D. (1995) Interlaboratory Comparison of Liver Spontaneous Mutant Frequency from Lambda/LacI Transgenic Mice (Big Blue®) (II). *Mutation Research* 327:67-73.
67. Callahan, J. and Short, J.M. (1995) Transgenic λ /lacI Mutagenicity Assay: Statistical Determination of Sample Size. *Mutation Research* 327:201-208.
68. Wyborski, D.L., Malkhosyan, S., Moores, J.C., Dyaico, M.J., Perucho, M. and Short, J.M. (1995) Development of a Rat Cell Line Containing a Lambda Shuttle Vector for *In Vitro* Mutagenicity Testing. *Mutation Research* 334:161-165.
69. Snead, M.A., Kretz, P.L., and Short, J.M. (1995) Methods for Generating Plant Genomic Libraries. *Plant Molecular Biology Manual*, Kluwer Academic Publishers: Belgium (H1, 1-19).
70. Robertson, D.E., Mathur, E.J., Swanson, R.V., Marrs, B.L., and Short, J.M. (1996) The Discovery of New Biocatalysts From Microbial Diversity. *SIM News* 46:3-8.
71. Knoll, A., Jacobson, D.P., Nishino, H., Kretz, P.L., Short, J.M., and Sommer, S. (1996) A Selectable System for Mutation Detection in the Big Blue® lacI Transgenic Mouse System: What Happens to the Mutational Spectrum Over Time. *Mutation Research* 352:9-22.
72. Wyborski, D.L., DuCoeur, L.C., and Short, J.M. (1996) The Effect of Chromosome Position and Operator Placement on Lac Repressor Control in Eukaryotic Cells and Transgenic Mice. *Environmental and Molecular Mutagenesis*. 28:447-458.
73. Sick, A.J., Fernandez J. and Short, J.M. (1996) Multiple Purpose Cloning Vectors. *Molecular Biology* (published).
74. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:39-51.
75. Snead, M., Alting-Mees, M.A., and Short, J.M. (1997) Clone Excision Methods for the Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, cDNA Library Protocols*. Humana Press 69:53-60.
76. Short, J.M. (1997) Recombinant Approaches for Accessing Biodiversity. *Nature Biotechnology* 15:1322-1323.
77. Nichols, W.S., Geller, S.A., Edmond, V.J., Dyaico, M.J., Sorge, J.A., and Short, J.M. (1998) Hepatocarcinogenesis (Z#2) / mutagenesis during initiation stage. *Mutation Research* 398:143-149.
78. Snead, M., Alting-Mees, M.A., and Short, J.M. (1998) cDNA Library Construction for Lambda ZAP® - Based Vectors. *Methods in Molecular Biology, Plant Virology Protocols*. Humana Press 81:255-267.
79. Bruggeman, T., Short, J.M., and Simms, P. (1998) Diversa: Catalyzing a Revolution. *Industrial Biotech News* 1(1):4,14-15.
80. Deckert, G., Warren, P.V., Gaasterland, T., Young, W.G., Lenox, A.L., Graham, D.E., Overbeek, R., Snead, M., Keller, M., Aujay, M., Huber, R., Feldman, R.A., Short, J.M., Olsen, G.J., and Swanson, R.V. (1998) The Complete Genome of the Hyperthermophilic Bacterium *Aquifex aeolicus*. *Nature* 392:353-358.
81. Li, J., Robertson, D.E., Short, J.M., Wang, P.G. (1999) Chemical and enzymatic synthesis of glycoconjugates. 5: One-pot regioselective synthesis of bioactive galactobiosides using a CLONEZYME thermophilic glycosidase library. *Bioorganic & Medicinal Chemistry Letters* Jan 4; 9 (1):35-8

82. Snead, M.A., Alting-Mees, M.A., Short, J.M. (2000) cDNA Library Construction for the Lambda ZAP® - Based Vectors. *Nucleic Acid Protocols Handbook, Humana Press Part V*:355-365.
83. Sehgal, A.C., Callen W., Mathur E. J., Short, J.M., Kelly, R.M. (2001) Carboxylesterase from *Sulfolobus Solfataricus* P1. *Methods in Enzymology* 330:461-471.
84. Cady, S.G., Bauer, M.W., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-Endoglucanase from *Pyrococcus Furiosus*. *Methods in Enzymology* 330:346-354.
85. Miller, E.S., Kimberley, Parker, N., Liebl, W., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Alpha-D-galactosidases from *Thermotoga* Species. *Methods in Enzymology* 330:246-260.
86. Chhabra, S., Parker, K.N., Lam, D., Callen, W., Snead, M.A., Mathur, E.J., Short, J.M., Kelly, R.M. (2001) Beta-mannanases from *Thermotoga* Species. *Methods in Enzymology* 330:224-238.
87. Parker, K.N., Chhabra, S.R., Lam, D., Callen, W., Duffaud, G.D., Snead, M.A., Short, J.M., Mathur, E.J., Kelly, R.M. (2001) Galactomannanases Man2 and Man5 from *Thermotoga* species: growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes. *Biotechnology and Bioengineering* Nov 5;75 (3):322-33
88. Gray, K.A., Richardson, T.H., Kretz, K., Short, J.M., Bartnek, F., Knowles, R., Kan, L., Swanson, P.E., Robertson, D.E. (2001) Rapid Evolution of Reversible Denaturation and Elevated Melting Temperature in a Microbial Haloalkane Dehalogenase. *Advanced Synthesis & Catalysis* 2001, 343:607-617.
89. Richardson, T.H., Tan, X., Frey, G., Callen, W., Cabell, M., Lam, D., Macomber, J., Short, J.M., Robertson, D., Miller, C. (2002) A Novel, High Performance Enzyme for Starch Liquefaction: Discovery and Optimization of a Low pH, Thermostable α -amylase. *Journal of Biological Chemistry* 2002, 277(29), 26501-26507.
90. Zengler, K., Toledo, G., Rappe, M., Elkins, J., Mathur, E.J., Short, J.M., Keller, M. (2002) Cultivating the Uncultured. *Proceedings of the National Academy of Sciences of the United States of America* (2002), 99(24), 15681-15686.
91. Murphy, K.M., Broman, K.W., Ziegler, J.S., Wyborski, D.L., Joe, L.K., Smith, D.W., Thurston, L.M., Stevenson, S.E., McClelland, M., Short, J.M., Mathur, E.J., Varley, J.D. (2002) Successful Breeding Among Free-Ranging 10-Month-Old Gray Wolves (*Canis Lupus*) in Yellowstone National Park, Wyoming. *Yellowstone Center for Resources* (2002). Kerry M. Murphy (kerry_murphy@nps.gov), P.O. Box 168, Yellowstone National Park, WY 82190.

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A \longrightarrow B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A \longrightarrow B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which comprises SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.